

S/N 09/096,749**PATENT****IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant: Shohei Koide

Examiner: Larry R. Helms, Ph.D.

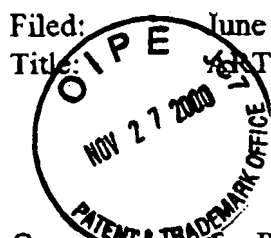
Serial No.: 09/096,749

Group Art Unit: 1642

Filed: June 12, 1998

Docket: 109.034US1

Title: ARTIFICIAL ANTIBODY POLYPEPTIDES

**Declaration of Shohei Koide under 37 C.F.R. § 1.132**Commissioner for Patents
Washington, D.C. 20231

I, Shohei Koide, Ph.D, declare and say as follows:

1. I am the inventor of the above-referenced U.S. Patent Application.
2. In addition to the examples presented in the original filing, the I have performed additional experiments that further support the pending claims. I prepared mutant FNfn10 proteins that contain glycine insertions in the BC, DE or FG loops. Glycines were chosen to be inserted because glycine insertions are generally highly destabilizing. Glycines have a high degree of conformational freedom (large entropy) in the unrestrained state. It is thus energetically unfavorable to restrain glycines by folding of a protein, because of a high entropic penalty.
3. Mutant proteins were prepared using the Kunkel mutagenesis and the conformational stability was determined using guanidine hydrochloride-induced denaturation, as described in (Koide, A., Bailey, C. W., Huang, X. & Koide, S. (1998) The fibronectin type III domain as a scaffold for novel binding proteins. *J. Mol. Biol.* 284, 1141-1151). The Figure attached to the present Declaration shows effects of these mutations on the conformational stability of FNfn10. Specifically, the Figure depicts denaturation curves of FNfn10 and its variants containing additional glycine residues in one or more loops. Denaturation reactions were monitored using tryptophan fluorescence and analyzed according to the two-state model, as described in Koide et al (1998). Experiments were performed in 10 mM sodium citrate buffer pH 6.0 containing 100 mM sodium chloride at 30 °C.
4. The following Table A summarizes the free energy of unfolding in the absence of guanidine hydrochloride.

#13 12/8/00
T. Gray



Page 2

Serial Number: 09/096,749

Dkt: 109.034US1

Filing Date: June 12, 1998

Title: ARTIFICIAL ANTIBODY POLYPEPTIDES

Table A: Effects of glycine insertions on the conformational stability of FNfn10

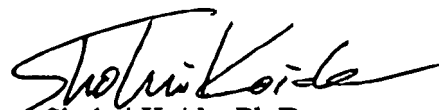
Protein	ΔG (kcal/mol)
Wild Type	7.70 ± 0.09
Four glycines in the FG loop	6.91 ± 0.15
Eight glycines in the FG loop	6.62 ± 0.11
Four glycines in the DE loop	5.66 ± 0.21
Four glycines in the BC loop and eight glycines in the FG loop	6.09 ± 0.17

These results show that these insertions are in fact destabilizing, but these mutant FNfn10 proteins are still highly stable. Thus, these results demonstrate that it is feasible to make mutant proteins in which the BC, DE and/or FG loops contain deletions, insertions and replacements.

5. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Date November 17, 2000

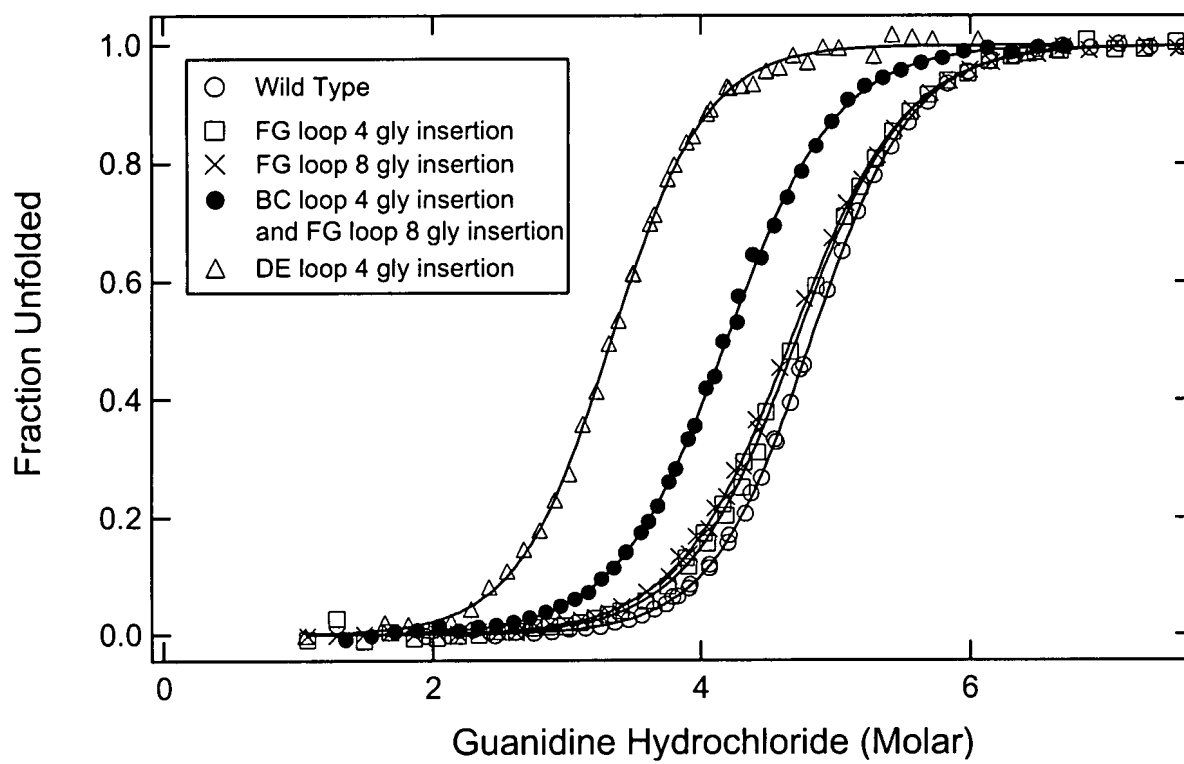
By


Shohei Koide, Ph.D.

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Commissioner of Patents, Washington, D.C. 20231, on November , 2000.

Name

Signature



S/N 09/096,749

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Shohei Koide

Examiner: Larry R. Helms,

Ph.D.

Serial No.: 09/096,749

Group Art Unit: 1642

Filed: June 12, 1998

Docket: 109.034US1

Title: ARTIFICIAL ANTIBODY POLYPEPTIDES



Declaration under 37 C.F.R § 132

Commissioner for Patents
Washington, D.C. 20231

I, Anne Koch, declare and say as follows:

1. I am in the employ of the law firm of Schwegman, Lundberg, Woessner & Kluth, P.A., 1600 TCF Tower, 121 South Eighth Street, Minneapolis, Minnesota 55402, Applicant's attorney of record in the above-identified patent application.

2. On September 15, 2000, I was informed by Sandra W. Jacobson, Senior Editor at *The FASEB Journal*, that volume 11, number 9 of *The FASEB Journal* was mailed from the printer on August 7, 1997. This volume was available to the public on the first day of the meeting, August 24, 1997. A copy of the e-mail communication that I received from Sandra W. Jacobson is attached as Exhibit A.

3. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Date 11-20-00 By Anne Koch
Anne Koch

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Commissioner of Patents, Washington, D.C. 20231, on November 20, 2000.

Candis B. Buending

Name

Signature



Ann Viksnins

From: Anne Koch
Sent: Monday, September 18, 2000 10:36 AM
To: Ann Viksnins
Subject: FW: Publication Availability Date (FASEBJ Feedback Form)

-----Original Message-----

From: Sandra W. Jacobson [mailto:sjacobson@faseb.org]
Sent: Friday, September 15, 2000 8:55 AM
To: koch@slwk.com
Cc: jborchar@faseb.org
Subject: Fwd: Publication Availability Date (FASEBJ Feedback Form)

Dear Ms Koch,

As you probably know, volume 11, number 9 of *The FASEB Journal* is a book of meeting abstracts, not full-length papers. Our records show that the abstract issue was mailed from our printer in Pennsylvania on August 7, 1997. The print version was available to the public on the first day of the meeting, August 24, 1997. Knowing when it went online is a little trickier. Our best guess is sometime around June 23. Abstracts that were submitted electronically, appeared online as complete abstracts; those submitted as hard copy appeared with titles and authors only. The abstracts are removed from online shortly after the end of the meeting.

I hope this is of some help.

Sincerely,

Sandra W. Jacobson
 Senior Editor
 The FASEB Journal

>
 >>From: "Anne Koch" <koch@slwk.com>
 >>To: fasebj-feedback@forsythe.stanford.edu, jborchar@faseb.org,
 >> nrodrnan@faseb.org
 >>Cc: koch@slwk.com
 >>Subject: Publication Availability Date (FASEBJ Feedback Form)
 >>Date: Thu, 14 Sep 2000 15:14:38 -0700
 >>X-Mailer: Highwire Mailer
 >>
 >>-----
 >>Comments sent via <|>FJ</|> Feedback Page
 >>-----
 >> NAME: Anne Koch
 >> EMAIL: koch@slwk.com
 >> IP ADDRESS: 216.70.219.130

9/18/00

EXHIBIT A

>> HOSTNAME: sw.slwk.com
>> PREVIOUS PAGE: <http://www.fasebj.org/misc/ifora-rescomm.shtml>
>> BROWSER: Mozilla/4.74 [en] (Win95; U)
>>-----
>>COMMENTS:
>>I need to find out when the following article was
>>publically available to your subscribers.
>>
>>When was it published? When was it mailed out to
>>your subscribers? When was it available online? When
>>was it available at the meeting?
>>
>>Thanks very much for your time.
>>Anne Koch
>>
>>2/3/1 (Item 1 from file: 5)
>>DIALOG(R)File 5:Biosis Previews(R)
>>(c) 2000 BIOSIS. All rts. reserv. 11099061 BIOSIS
>>NO.: 199799720206
>>
>>Directed evolution of fibronectin type III domain
>>to novel ligand binding proteins.
>>AUTHOR: Koide Shohei; Bailey Charles W; Koide Akiko
>>AUTHOR ADDRESS: Dep. Biochem. and Biophysics, Univ.
>>Rochester Med. Cent., Rochester, NY 14642**USA
>>1997
>>JOURNAL: FASEB Journal 11 (9):pA1155 1997
>>CONFERENCE/MEETING: 17th International Congress of
>>Biochemistry and Molecular Biology in conjunction with
>>the Annual Meeting of the American Society for
>>Biochemistry and Molecular Biology San Francisco,
>>California, USA August 24-29, 1997
>>ISSN: 0892-6638
>>RECORD TYPE: Citation
>>LANGUAGE: English
>
>-----
>La Vonne Gallo
> HighWire Press, Stanford University
> <http://highwire.stanford.edu/~lgallo>
> Phone: 650.725.2067/Fax:650.725.9335
>
>lgallo@highwire.stanford.edu
>-----
>

• Sandra W. Jacobson
Senior Editor
The FASEB Journal
9650 Rockville Pike
Bethesda, MD 20814-3998
Ph: 301-530-7104
Fax: 301-571-1855
E-mail: sjacobson@faseb.org